

What is claimed is:

1. A recombinant LSA-NRC polypeptide comprising at least one LSA-1 epitope.

2. A recombinant LSA-NRC polypeptide according to claim 1 wherein said polypeptide comprises any of the epitopes defined by

(i) codons encoding *P. falciparum* LSA-1 N-terminal;

(ii) codons encoding *P. falciparum* LSA-1 C-terminal;

(iii) one or more 17 amino acid repeat unit

10 GluGlnGlnSerAspLeuGluGlnGluArgLeuAlaLysGluLysLeuGln
(SEQ ID NO:1);

(iv) one or more amino acid repeat unit

GluGlnGlnArgAspLeuGluGlnGluArgLeuAlaLysGluLysLeuGln
(SEQ ID NO:2);

15 (v) one or more amino acid repeat unit following the order: X₁GlnGlnX₂AspX₃GluGlnX₄ArgX₅AlaX₆GluX₇LeuGln
(SEQ ID NO:5) where x₁ is either Glu or Gly; x₂ is

Ser or Arg; x₃ is Asp or Ser; x₄ is Glu or Asp; x₅ is Leu or Arg; x₆ is Lys or Asn and x₇ is Lys or Thr or

20 Arg; and

(vi) one or more epitope specified in SEQ ID NO:6-23.

3. A polypeptide according to claim 2 wherein said polypeptide is harmonized.

25 4. The polypeptide of claim 3 wherein said polypeptide is LSA-NRC(H) specified in SEQ ID NO:26.

5. The LSA-NRC(H) polypeptide according to claim 3 further comprising a mutation in the T5 epitope, LSA-NRC(H)Mut, specified in SEQ ID NO:4.

30 . 6. A composition comprising the recombinant *P. falciparum* LSA-NRC of claim 1.

7. A composition comprising the recombinant polypeptide of claim 2.

8. A composition comprising the recombinant polypeptide of claim 3.

9. A composition comprising the recombinant polypeptide of claim 4.

5 10. A composition comprising the recombinant polypeptide of claim 5.

11. A recombinant vector comprising a DNA sequence encoding LSA-NRC according to claim 1.

10 12. A recombinant vector comprising a DNA sequence encoding LSA-NRC according to claim 2.

13. A recombinant vector comprising a DNA sequence encoding LSA-NRC(H) according to claim 4.

15 14. A recombinant vector comprising a DNA sequence encoding LSA-NRC(H)Mut according to claim 5.

15. A recombinant vector comprising a DNA sequence encoding LSA-NRC according to claim 3.

16. The vector of claim 12 wherein said DNA sequence corresponds to SEQ ID NO:25.

20 17. The vector of claim 13 wherein said DNA sequence corresponds to SEQ ID NO:3.

18. The vector of claim 16 wherein said vector is pETK(-).

25 19. The vector of claim 17 wherein said vector is pETK(-).

20. The vector of claim 19 wherein said vector is pET KLSA-NRC^{hmut}.

21. A host cell transformed with the vector according to claim 18.

30 22. A host cell transformed with the vector according to claim 20.

23. The host cell of claim 20 wherein said host is *E. coli* Tuner (DE3).

24. A method for producing and purifying recombinant *P. falciparum* LSA-NRC polypeptide comprising:

- 5 (i) growing a host cell containing a vector expressing *P. falciparum* LSA-NRC polypeptide in a suitable culture medium,
 - (ii) causing expression of said vector under suitable conditions for production of soluble LSA-NRC polypeptide and,
 - 10 (iii) lysing said host cells and recovering said LSA-NRC polypeptide such that it retains its native folding.
25. The method of claim 24 further comprising removal of *E. coli* endotoxin.
- 15 26. The method of claim 25 wherein said removal of endotoxin is by
- (i) application of the lysed bacteria to a resin containing Ni-NTA and washing said resin bound material with low pH, high salt buffer,
 - 20 (ii) removal of bound material from Ni-NTA resin and binding to other ion affinity resins such as DEAE and SP-Sepharose resins such that the LSA-NRC polypeptide binds and the endotoxins can be washed away.
- 25 27. An antibody produced against the recombinant LSA-NRC polypeptide of claim 1.
28. An antibody produced against the recombinant LSA-NRC polypeptide of claim 2.
- 29 An antibody produced against the recombinant
- 30 LSA-NRC(H) polypeptide of claim 4.
30. An antibody produced against the recombinant LSA-NRC(H)Mut polypeptide of claim 5.
31. An antibody produced against the recombinant LSA-NRC polypeptide of claim 3.

32. The antibody of claim 27 wherein said antibody is monoclonal or polyclonal.

33. The antibody of claim 28 wherein said antibody is monoclonal or polyclonal.

5 34. The antibody of claim 29 wherein said antibody is monoclonal or polyclonal.

35. The antibody of claim 30 wherein said antibody is monoclonal or polyclonal.

10 36. The antibody of claim 31 wherein said antibody is monoclonal or polyclonal.

37. A method for *in vitro* diagnosis or detection of malaria antigen present in a biological sample, comprising:

15 (i) contacting said biological sample with a LSA-NRC specific antibody according to claim 27, preferably in an immobilized form under appropriate conditions which allow the formation of an immune complex,

(ii) removing unbound components,

20 (iii) incubating the immune complexes formed with heterologous antibodies which specifically bind to the antibodies present in the sample to be analyzed, with said heterologous antibodies conjugated to a detectable label under appropriate conditions,

(iv) detecting the presence of said immune complexes visually or mechanically.

38. A kit for *in vitro* detection of a malaria antigen present in a biological sample, comprising:

30 (i) at least one antibody which reacts with recombinant LSA-NRC according to claim 27, said antibody being preferentially immobilized on a solid substrate,

(ii) a buffer, or components necessary for producing the buffer, enabling binding reaction between these antibodies and the malaria antigens present in the biological sample, and

5 (iii) a means for detecting the immune complexes formed in the preceding binding reaction.

39. A recombinant protein according to any one of claims 1-5, wherein said purified protein is at least 90% pure.

10 40. An immunogenic carrier comprising a polypeptide according to claim 1.

41. An immunogenic carrier comprising a polypeptide according to claim 2.

15 42. An immunogenic carrier comprising a polypeptide according to claim 4.

43. An immunogenic carrier comprising a polypeptide according to claim 5.

44. An immunogenic carrier comprising a polypeptide according to claim 3.

20 45. A method for *in vitro* diagnosis of malaria antibodies in a biological sample, comprising

(i) contacting said biological sample with a composition comprising a LSA-NRC polypeptide according to claim 1 under appropriate conditions
25 which allow the formation of an immune complex, wherein said peptide is labeled with a detectable label, and

(ii) detecting the presence of said immune complexes visually or mechanically.

30 46. A kit for determining the presence of malaria antibodies in a biological sample, comprising:

(i) at least one polypeptide or protein composition according to claim 9,

a buffer or components necessary for producing a buffer;

(ii) means for detecting immune complexes formed between the peptide and antibodies present in
5 the sample.

47. A kit for determining the presence of malaria antibodies in a biological sample, comprising:

(i) at least one polypeptide or protein
10 composition according to claim 10,

a buffer or components necessary for producing a buffer;

(ii) means for detecting immune complexes formed between the peptide and antibodies present in
15 the sample.

48 A method for *in vitro* monitoring malaria infection or prognosing the response to treatment of patients suffering from malaria infection comprising:

(i) incubating a biological sample from a patient with malaria infection with an LSA-NRC protein according to claim 1 or a suitable part thereof under conditions allowing the formation of an immunological complex,
20

(ii) removing unbound components, calculating the anti-LSA-1 titers present in said sample.

49. A kit for monitoring malaria infection or prognosing the response to treatment of patients suffering from malaria infection comprising:

(i) at least one LSA-NRC peptide according to claim 1,
30

(ii) a buffer or buffer components,

(iii) means for detecting the immune complexes formed between the peptide and antibodies present in the sample, and

(iv) optionally, a means for determining the
5 amount of immune complex formed.

50. An immunogenic composition comprising *P. falciparum* LSA-NRC of claim 1.

51. An immunogenic composition comprising the polypeptide according to claim 2.

10 52. An immunogenic composition comprising the polypeptide according to claim 4.

53. An immunogenic composition comprising the polypeptide according to claim 5.

15 54. An immunogenic composition comprising the polypeptide according to claim 3.

55. The immunogenic composition of claim 50 further comprising an adjuvant.

56. The immunogenic composition of claim 51 further comprising an adjuvant.

20 57. The immunogenic composition of claim 52 further comprising an adjuvant.

58. The immunogenic composition of claim 53 further comprising an adjuvant.

25 59. The immunogenic composition of claim 54 further comprising an adjuvant.

60. The immunogenic composition of claim 55 wherein said adjuvant is chosen from the group consisting of: Montanide and alum.

30 61. The immunogenic composition of claim 56 wherein said adjuvant is chosen from the group consisting of: Montanide and alum.

62. The immunogenic composition of claim 57 wherein said adjuvant is chosen from the group consisting of: Montanide and alum.

63. The immunogenic composition of claim 58 wherein said adjuvant is chosen from the group consisting of: Montanide and alum.

5 64. The immunogenic composition of claim 59 wherein said adjuvant is chosen from the group consisting of: Montanide and alum.

65. A method for inducing in a subject an immune response against malaria infection comprising administering to said subject a composition
10 comprising an immunologically effective amount of *P. falciparum* LSA-NRC of claim 1 in an acceptable diluent.

66. The method of claim 65 wherein said composition further comprises an adjuvant.

15 67. The composition of claim 66 wherein said adjuvant is selected from the group consisting of Montanide, and alum.

68. A method for inducing in a subject an immune response against malaria infection comprising
20 administering to said subject a composition comprising an immunologically effective amount of *P. falciparum* LSA-NRC of claim 2 in an acceptable diluent.

25 69. The method of claim 68 wherein said composition further comprises an adjuvant.

70. The composition of claim 69 wherein said adjuvant is selected from the group consisting of Montanide, and alum.

71. A method for inducing in a subject an
30 immune response against malaria infection comprising administering to said subject a composition comprising an immunologically effective amount of *P.*

falciparum LSA-NRC of claim 3 in an acceptable diluent.

72. The method of claim 71 wherein said composition further comprises an adjuvant.

5 73. The composition of claim 72 wherein said adjuvant is selected from the group consisting of Montanide, and alum.

74. A method for inducing in a subject an immune response against malaria infection comprising
10 administering to said subject a composition comprising an immunologically effective amount of *P. falciparum* LSA-NRC of claim 4 in an acceptable diluent.

75. The method of claim 74 wherein said
15 composition further comprises an adjuvant.

76. The composition of claim 75 wherein said adjuvant is selected from the group consisting of Montanide, and alum.

77. A method for inducing in a subject an
20 immune response against malaria infection comprising administering to said subject a composition comprising an immunologically effective amount of *P. falciparum* LSA-NRC of claim 5 in an acceptable diluent.

25 78. The method of claim 77 wherein said composition further comprises an adjuvant.

79. The composition of claim 78 wherein said adjuvant is selected from the group consisting of Montanide, and alum.

30 80. A method for inducing a protective immune response to malaria in a mammal, comprising administering a composition comprising a *P. falciparum* LSA-NRC according to claim 1 in an amount

effective to induce an immune response in said mammal.

81. The method according to claim 80 wherein the composition further comprises an adjuvant
5 selected from the group consisting of Montanide, and alum.

82. A method for inducing a protective immune response to malaria in a mammal, comprising administering a composition comprising a *P. falciparum* LSA-NRC according to claim 2 in an amount
10 effective to induce an immune response in said mammal.

83. The method according to claim 82 wherein the composition further comprises an adjuvant
15 selected from the group consisting of Montanide, and alum.

84. A method for inducing a protective immune response to malaria in a mammal, comprising administering a composition comprising a *P. falciparum* LSA-NRC according to claim 3 in an amount
20 effective to induce an immune response in said mammal.

85. The method according to claim 84 wherein the composition further comprises an adjuvant
25 selected from the group consisting of Montanide, and alum.

86. A method for inducing a protective immune response to malaria in a mammal, comprising administering a composition comprising a *P. falciparum* LSA-NRC according to claim 4 in an amount
30 effective to induce an immune response in said mammal.

87. The method according to claim 86 wherein the composition further comprises an adjuvant selected from the group consisting of Montanide, and alum.

5 88. The method according to claim 84 wherein the composition further comprises an adjuvant selected from the group consisting of Montanide, and alum.

10 89. A method for inducing a protective immune response to malaria in a mammal, comprising administering a composition comprising a *P. falciparum* LSA-NRC according to claim 5 in an amount effective to induce an immune response in said mammal.

15 90. The method according to claim 89 wherein the composition further comprises an adjuvant selected from the group consisting of Montanide, and alum.

20 91. A multivalent vaccine for protection against infection with more than one strain of *P. falciparum*, said vaccine comprising LSA-NRC polypeptides from more than one strain of *P. falciparum* chosen from the group consisting of: 3D7, FVO, T9/96, NF54, and (what about other
25 strains)camp.

92. The multivalent vaccine of claim 91, further comprising an adjuvant selected from the group consisting of Montanide, and alum.